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Saposin C levels in plasma from control and LSD individuals

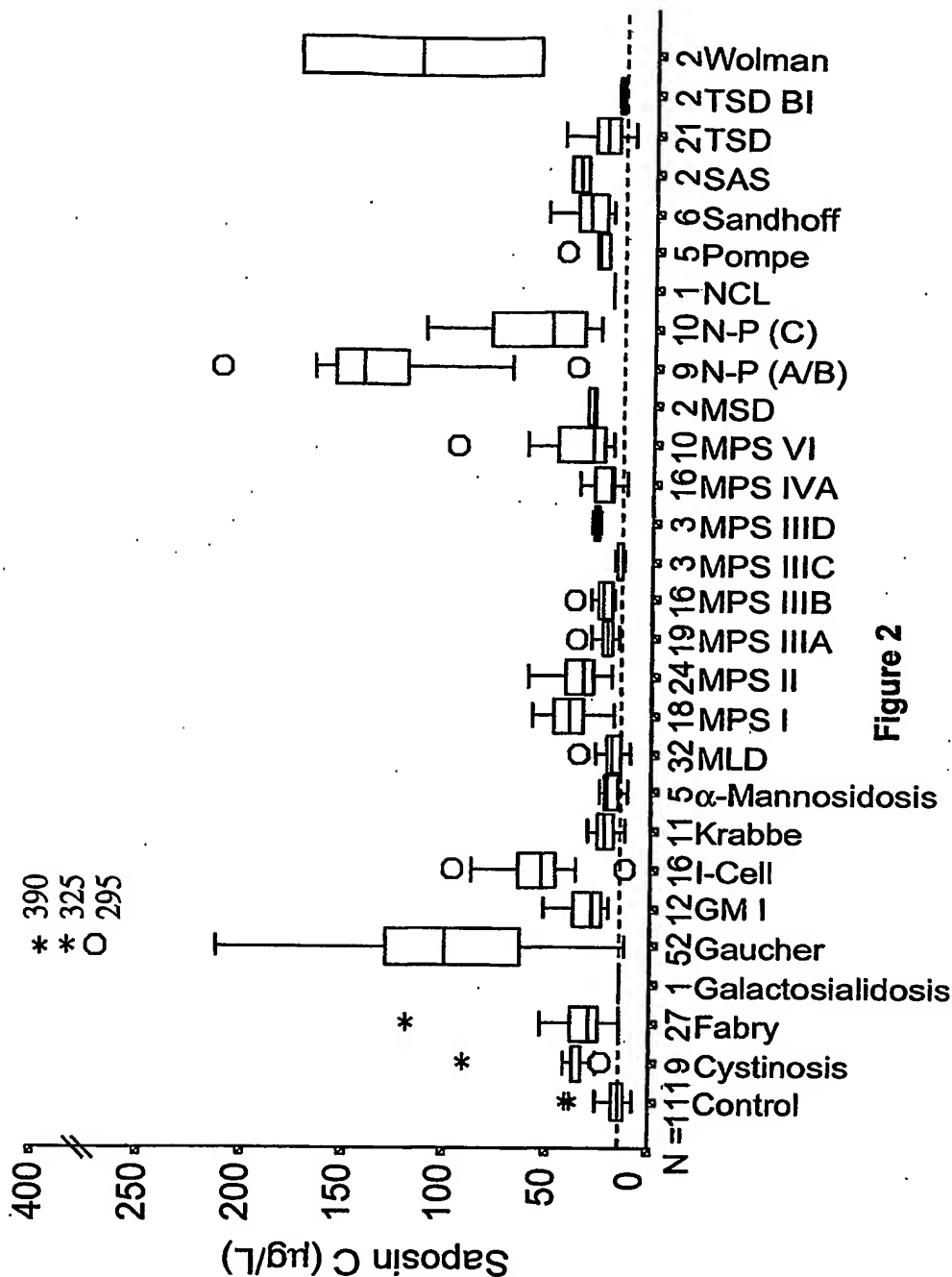


Figure 2

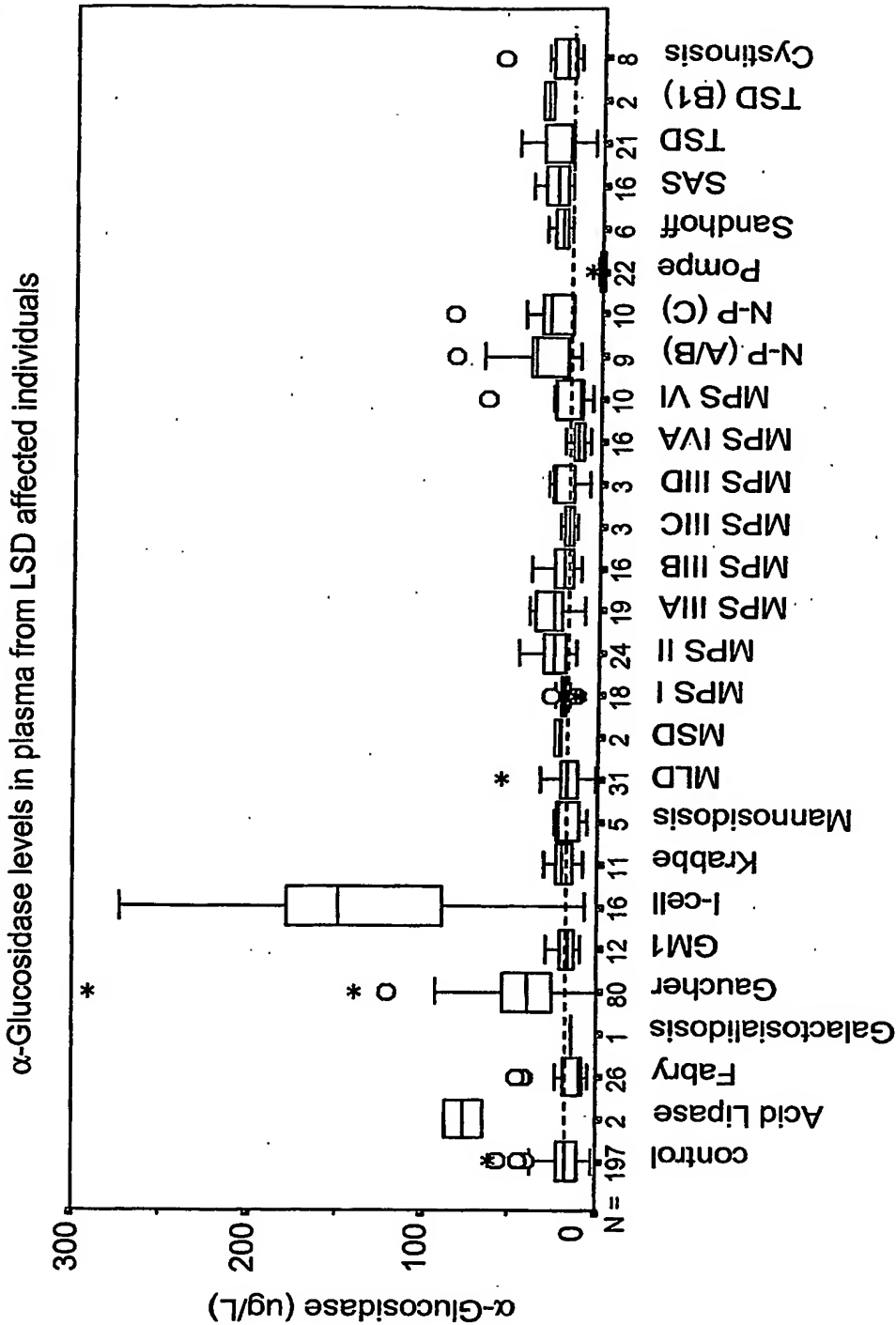


Figure 3

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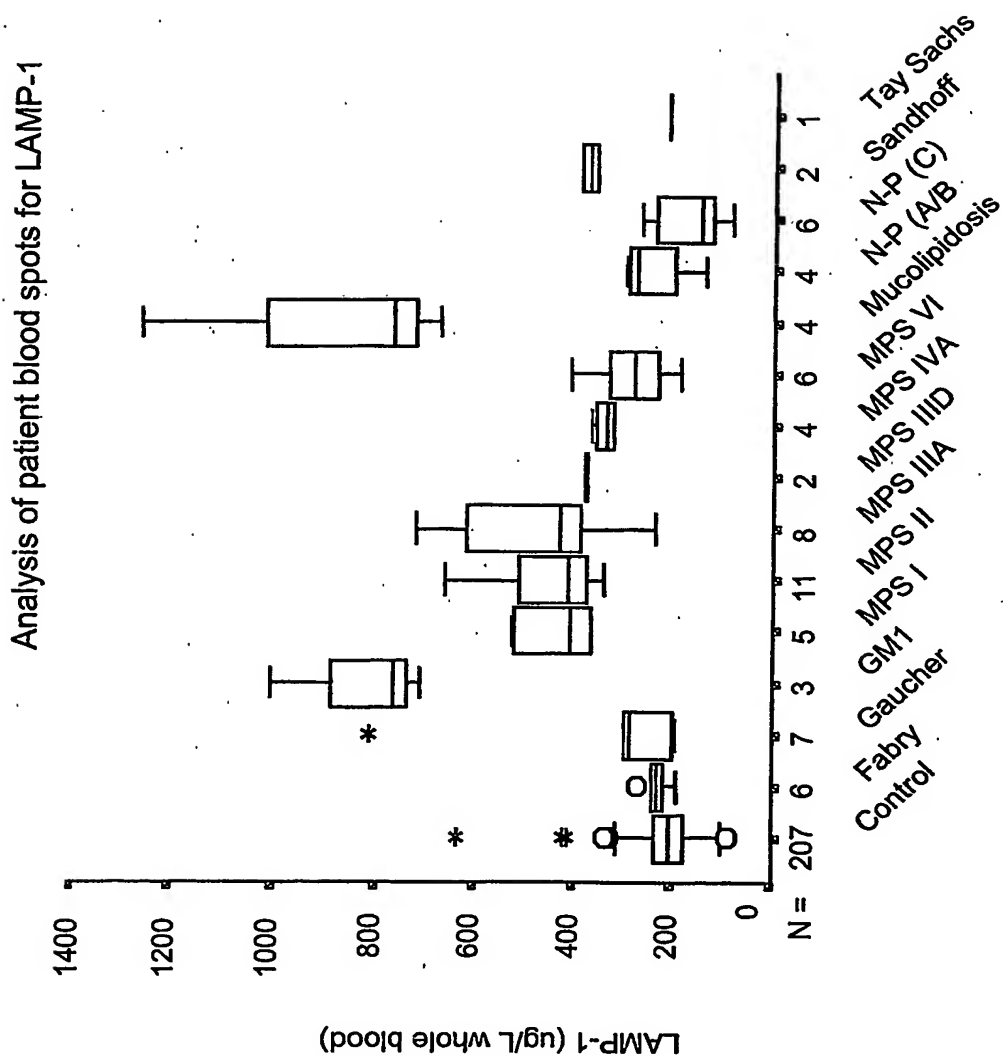


Figure 4

Analysis of patient blood spots for saposin C

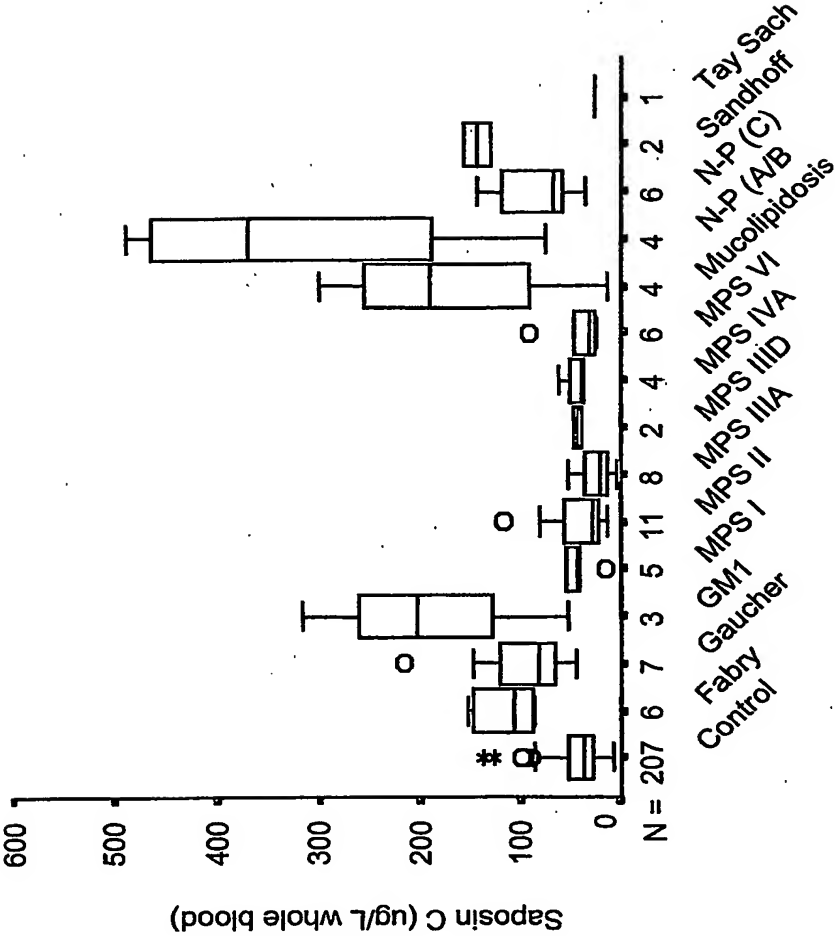


Figure 5

α -Glucosidase protein/activity determination
in dried blood spots

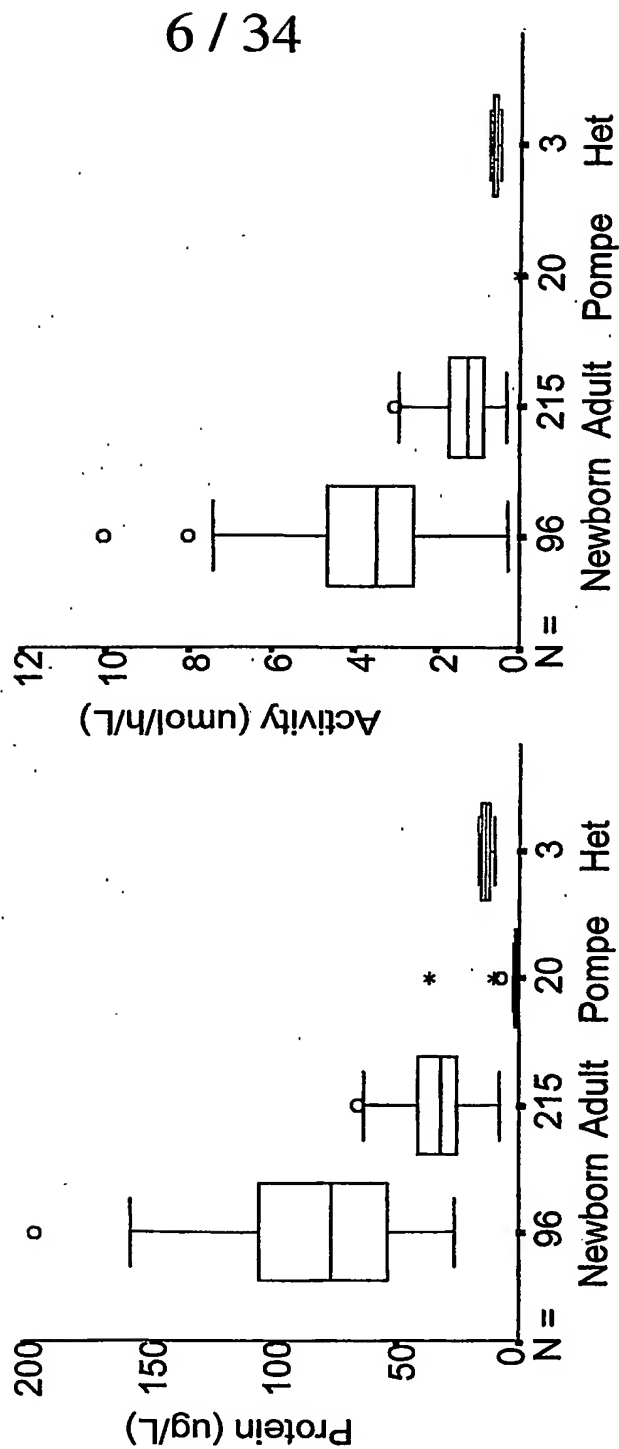


Figure 6

α -Glucosidase protein distribution in neonates

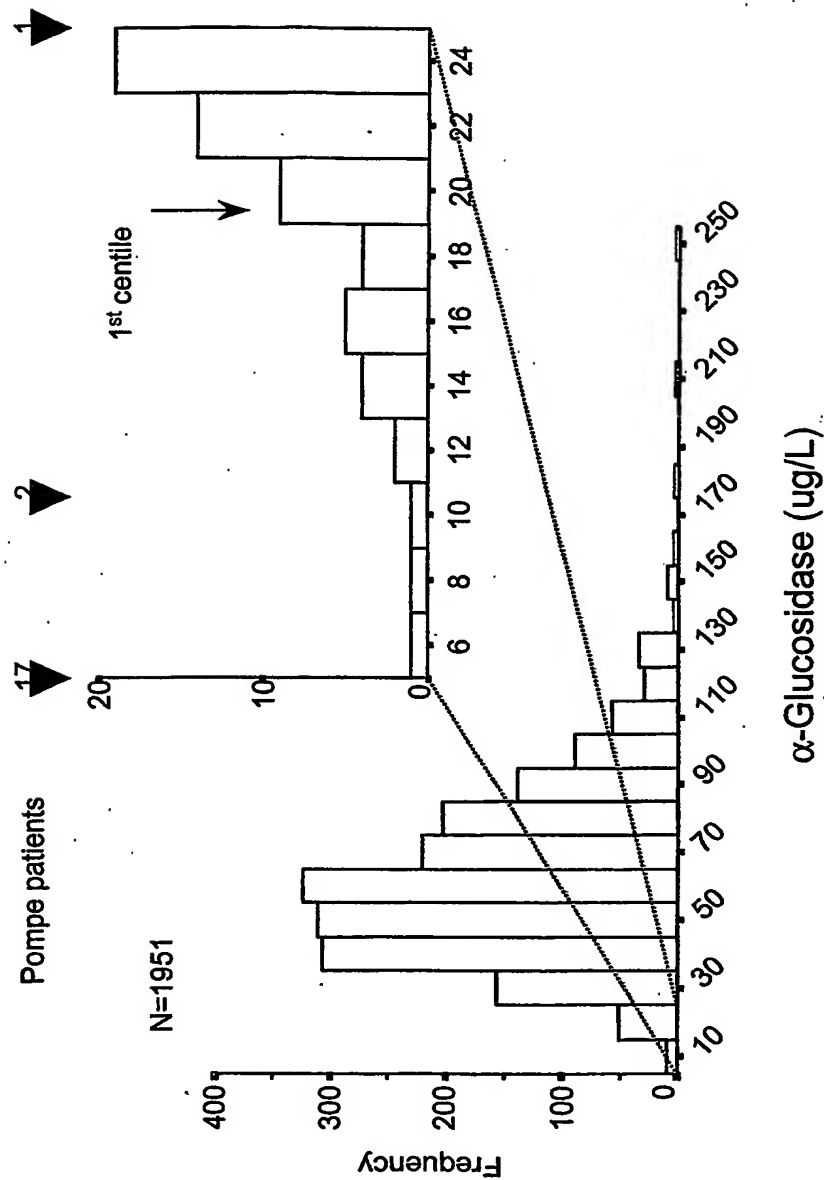


Figure 7

LAMP-1 & saposin C newborn population distribution
Dual TRFIMA assay for LAMP-1/saposin C

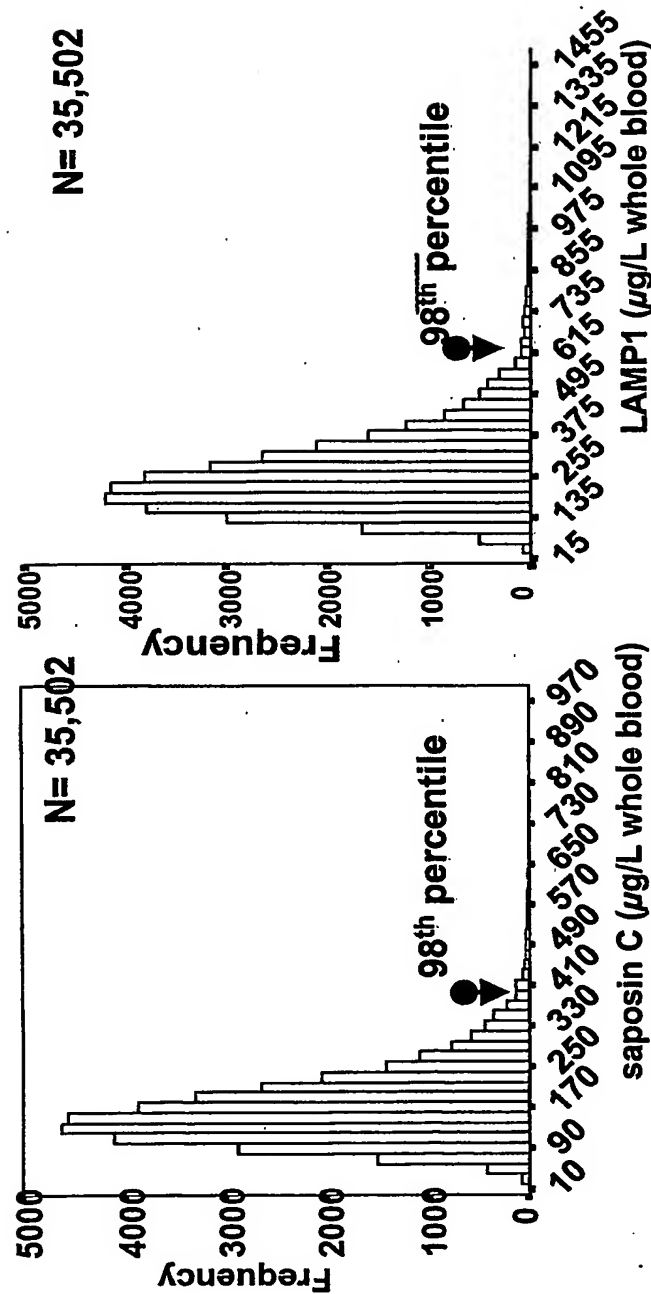


Figure 8

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Correlation between LAMP-1 and saposin C (N = 35,502)

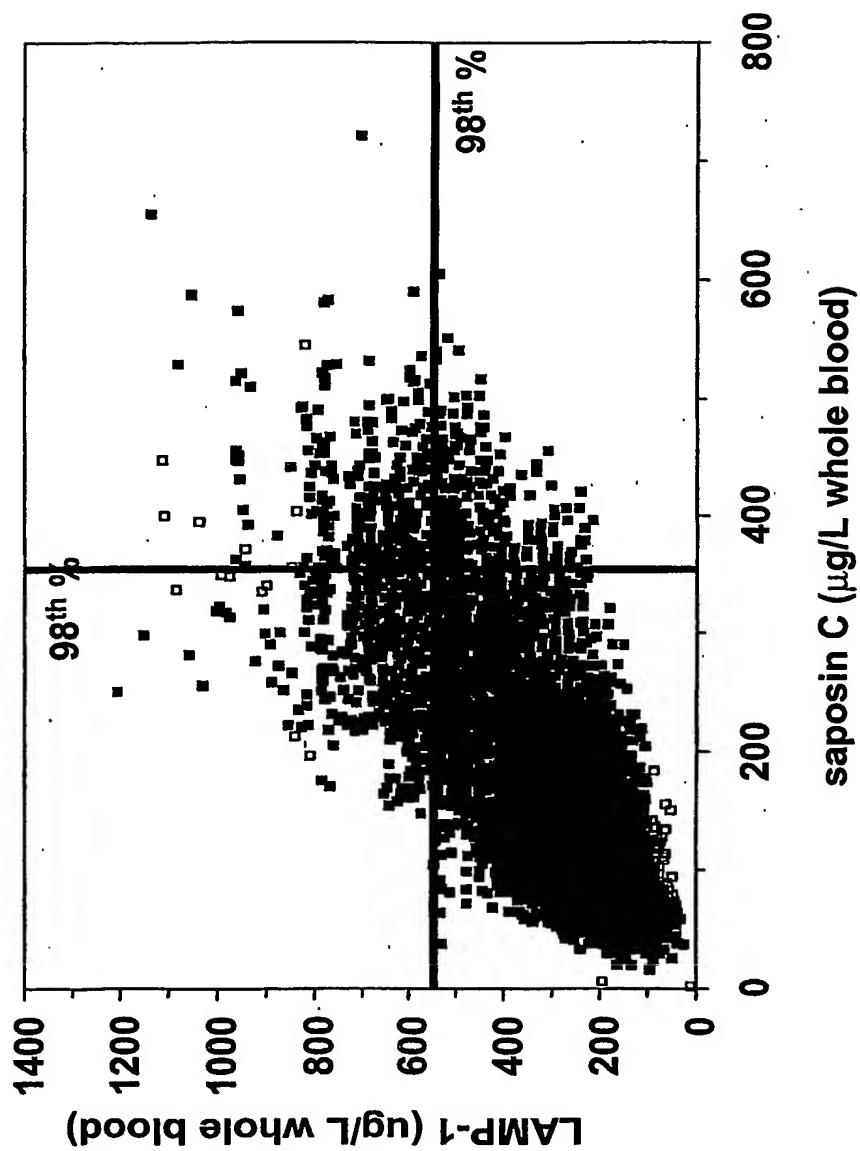


Figure 9

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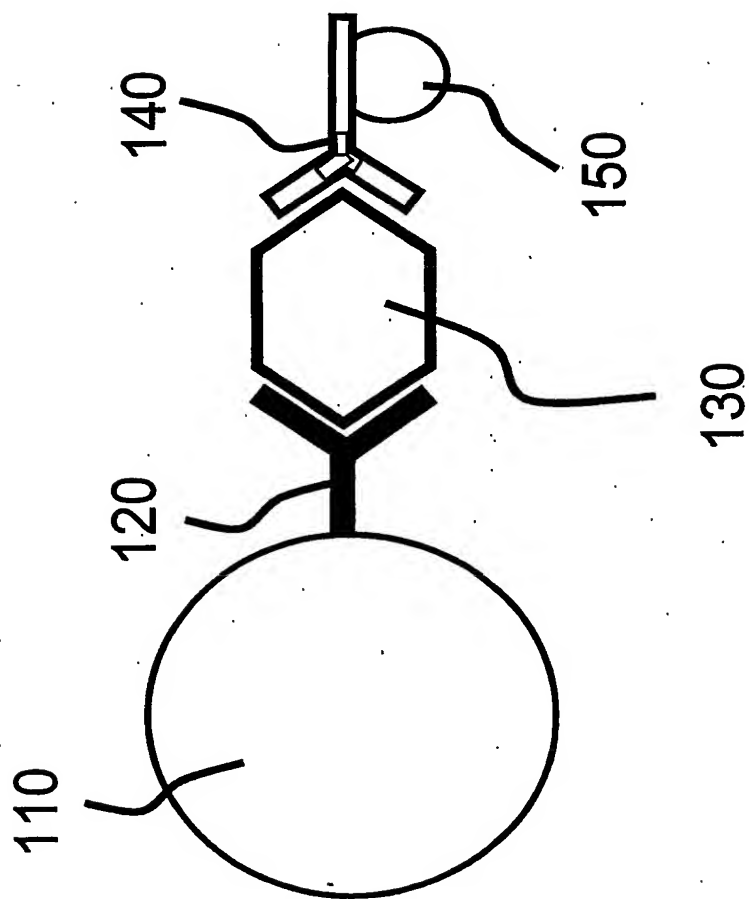


Figure 10

Antibody reagents available for lysosomal proteins

Priority	Disorder	Enzyme /Protein Marker	Protein	Polyclonal	Monoclonal (Complementary)
1		LAMP-1	CHO ex	Sheep	2
2		Saposin C		Rabbit	2
3		CD 45	commercial		
4	MPS I	α -L-iduronidase	CHO ex	Sheep	1
5	Pompe disease	α -glucosidase	CHO ex	Sheep	1
6	Gaucher disease	β -glucosidase	commercial	Sheep	1
7	Fabry disease	α -galactosidase A	commercial	Sheep	2
8	MPS VI	N-acetylglucosamine 4-sulphatase	CHO ex	Sheep	1
9	Niemann-Pick A/B	acid sphingomyelinase	commercial	Sheep	2
10	MPS II	iduronate-2-sulphatase	CHO ex	Sheep	
11	MPS IVA	galactose 6-sulphatase	CHO ex	Rabbit	
12	MLD	arylsulphatase A	CHO ex	Sheep	
13	Krabbe disease	galactocerebrosidase			
14	MPS IIIA	heparan-N-sulphatase	CHO ex	Rabbit	1
15	MPS IIIB	α -N-acetylglucosaminidase	CHO ex	Rabbit	

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Figure 11

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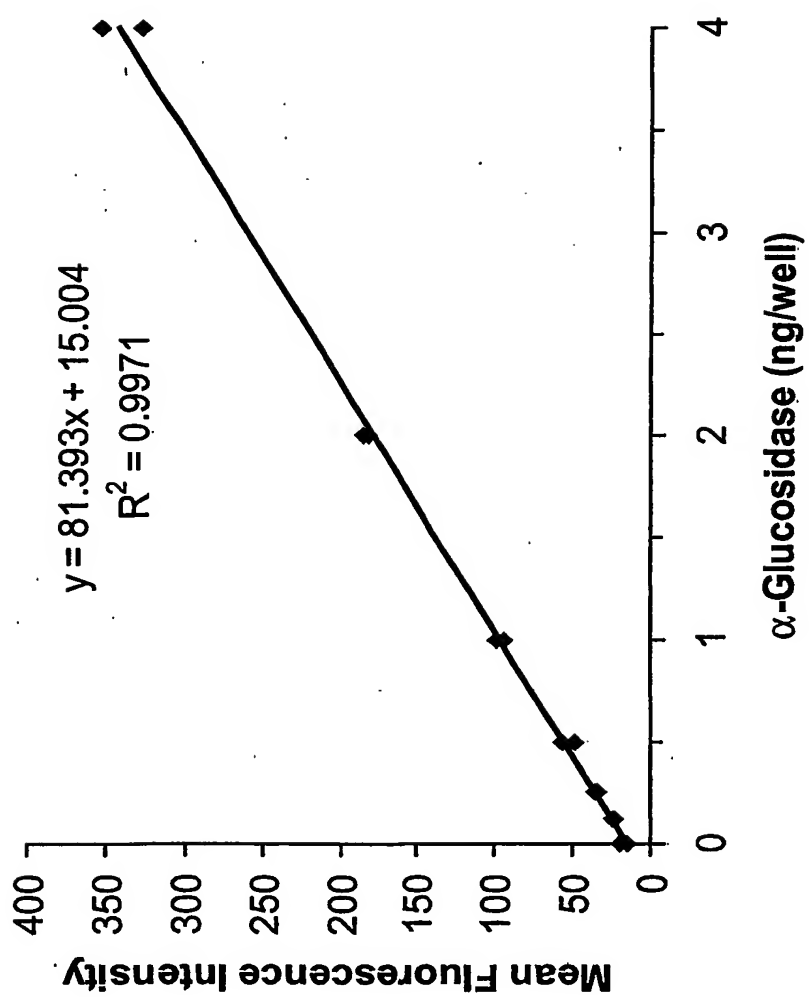


Figure 12

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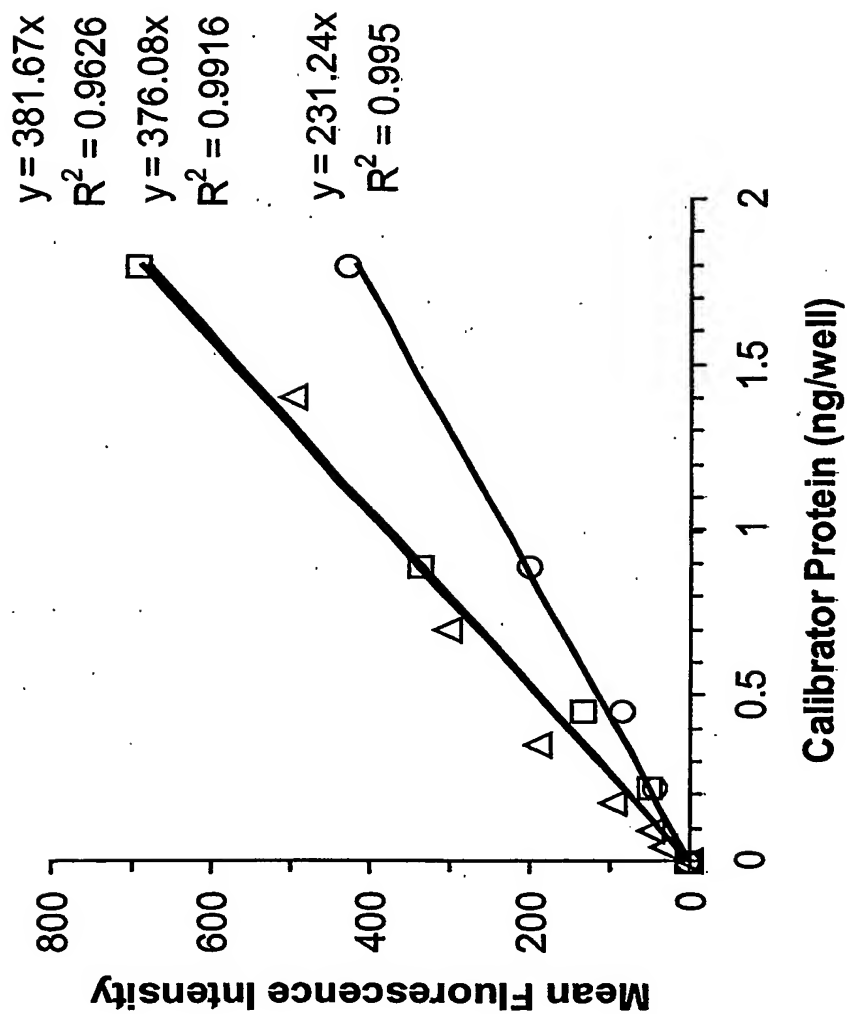


Figure 13

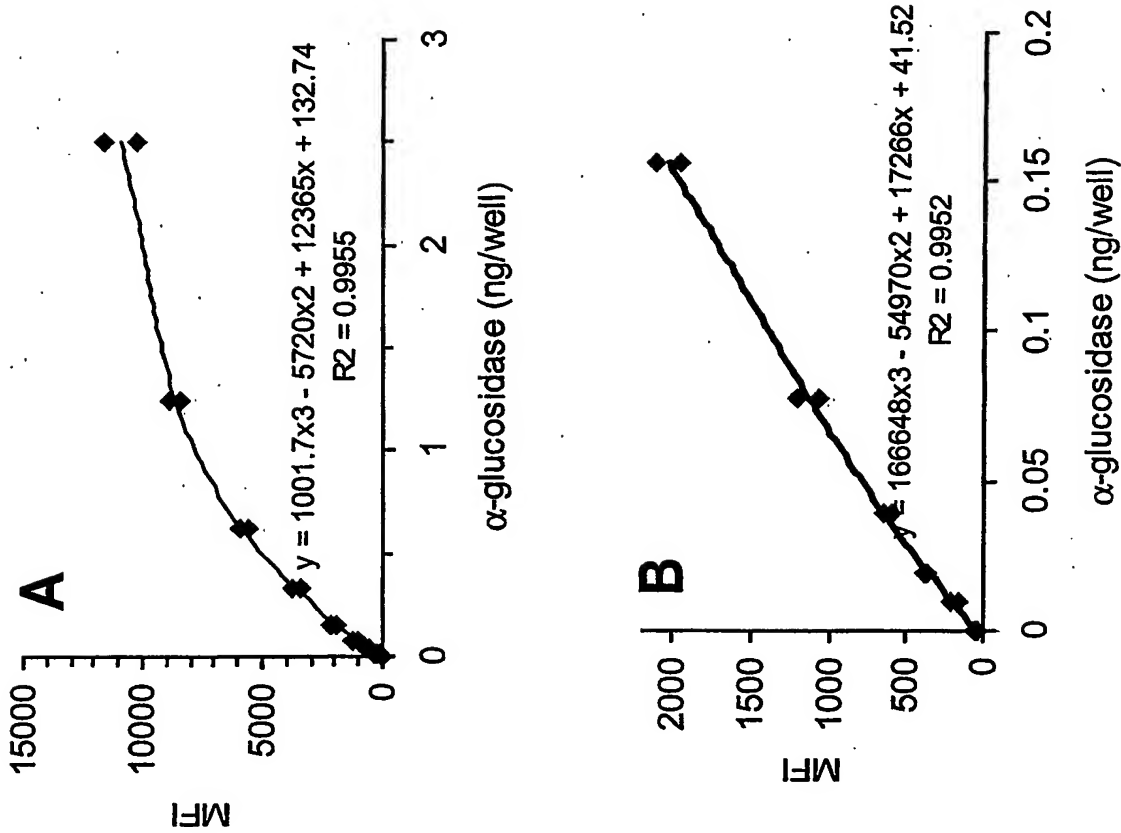
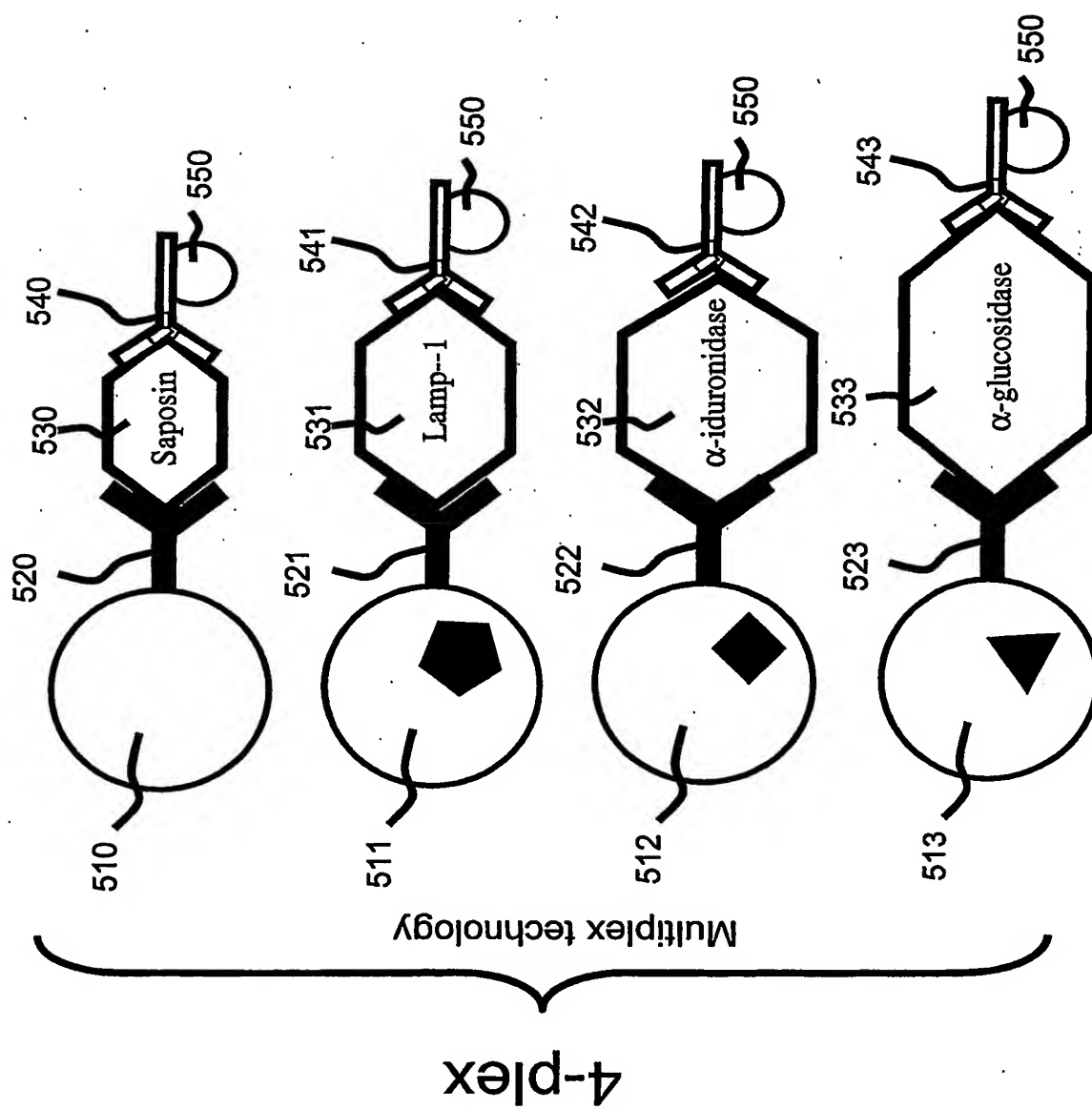


Figure 14

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Figure 15



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Multiplex calibration curves: 4-plex

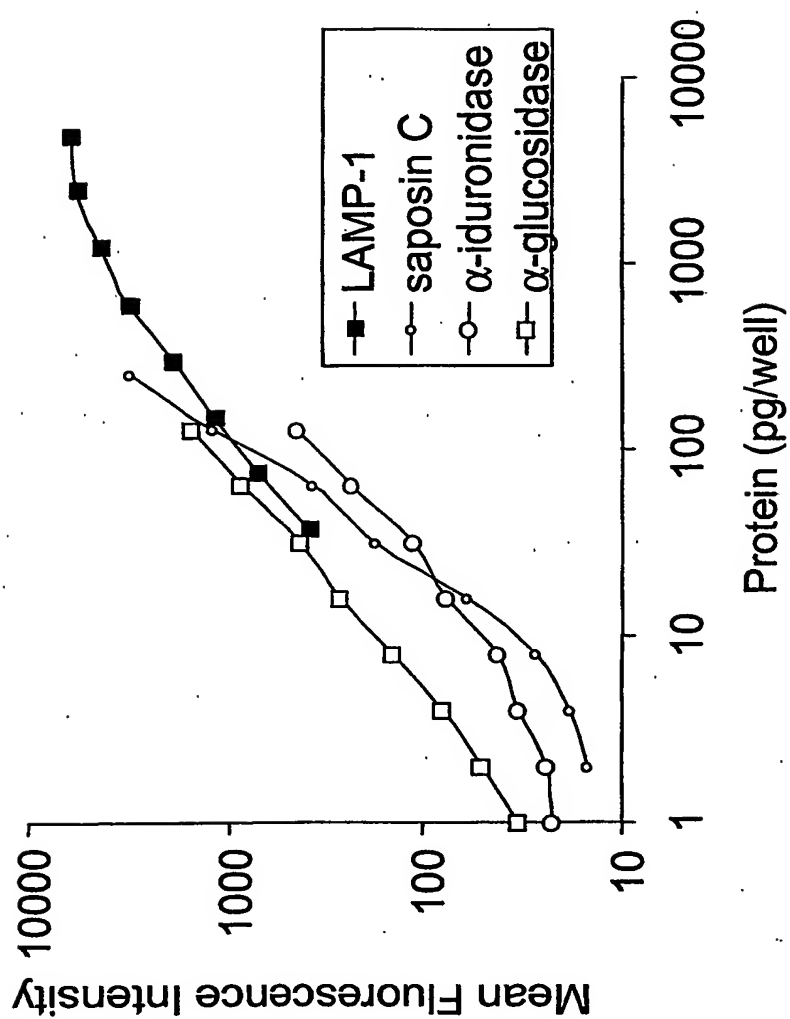


Figure 16

Multiplex analysis of control and MPS I plasma

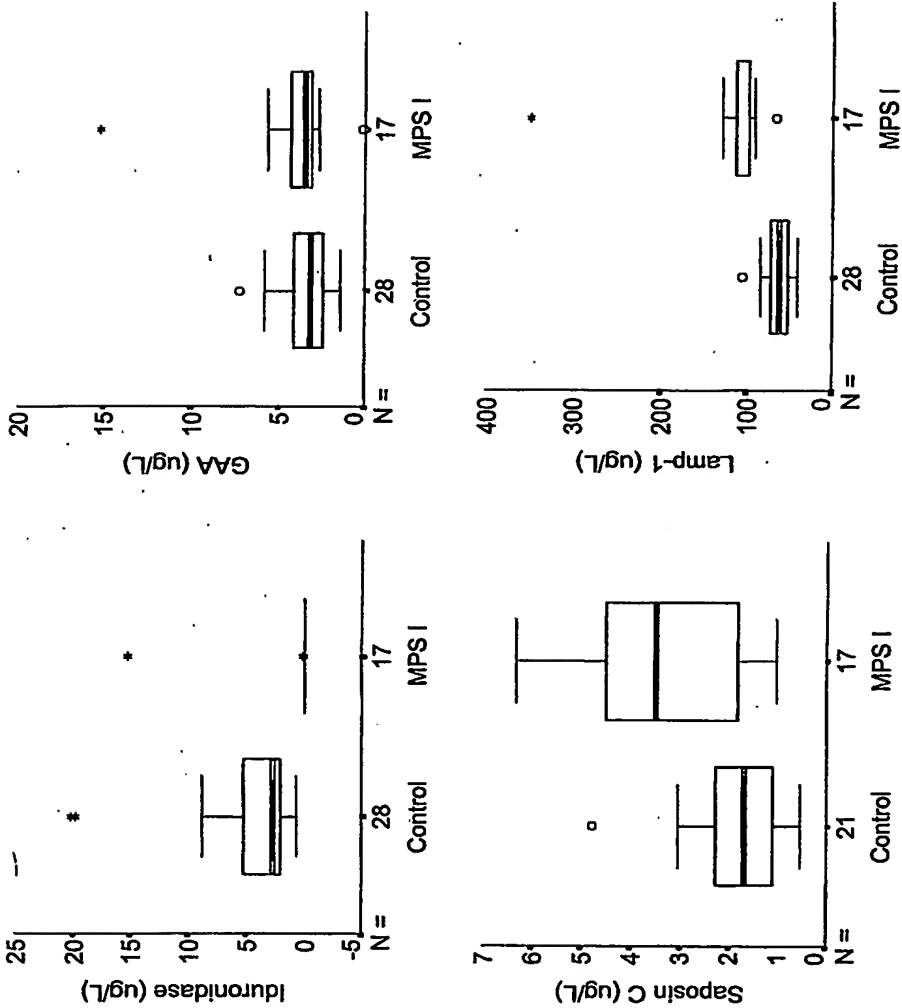


Figure 17

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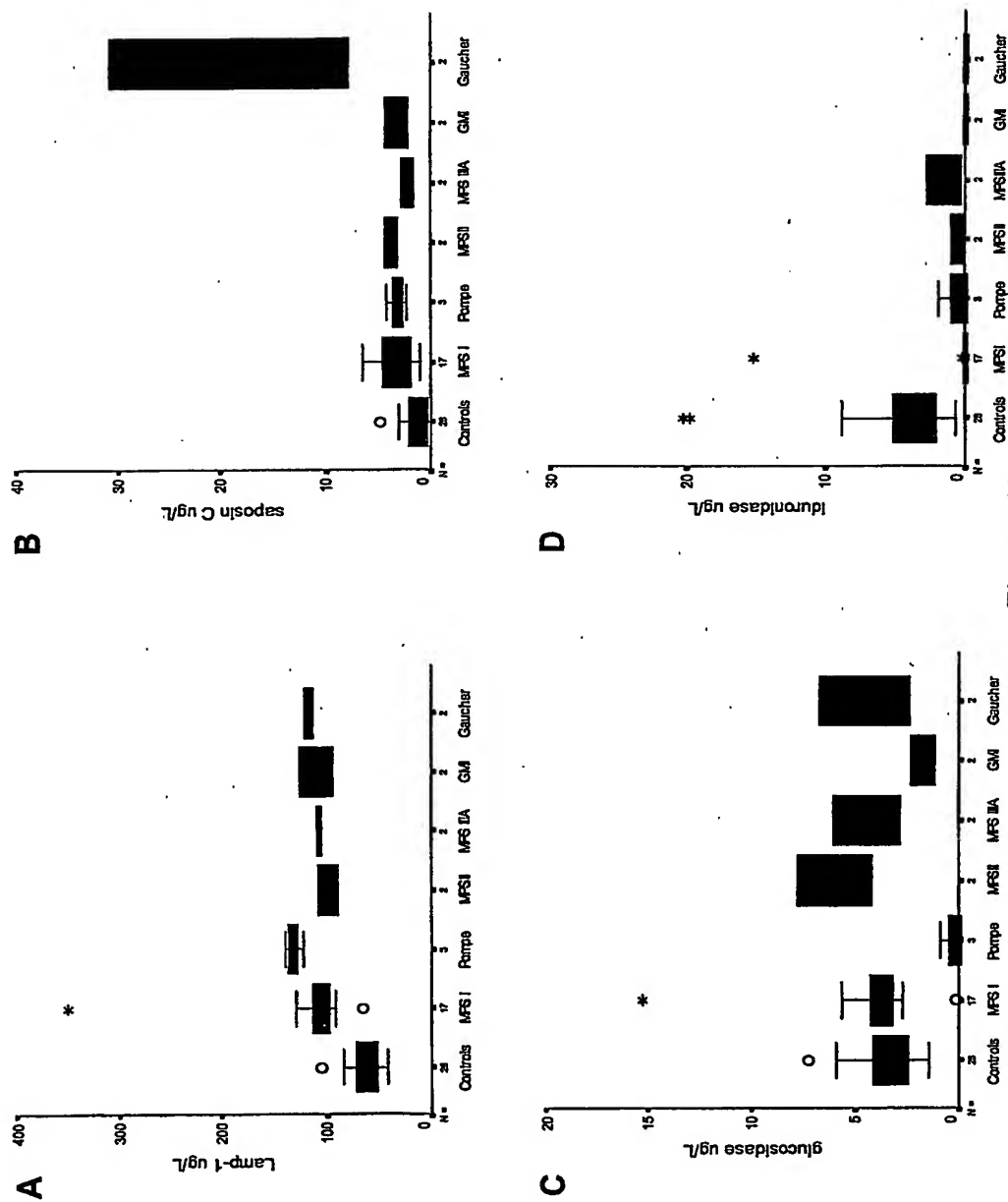


Figure 18

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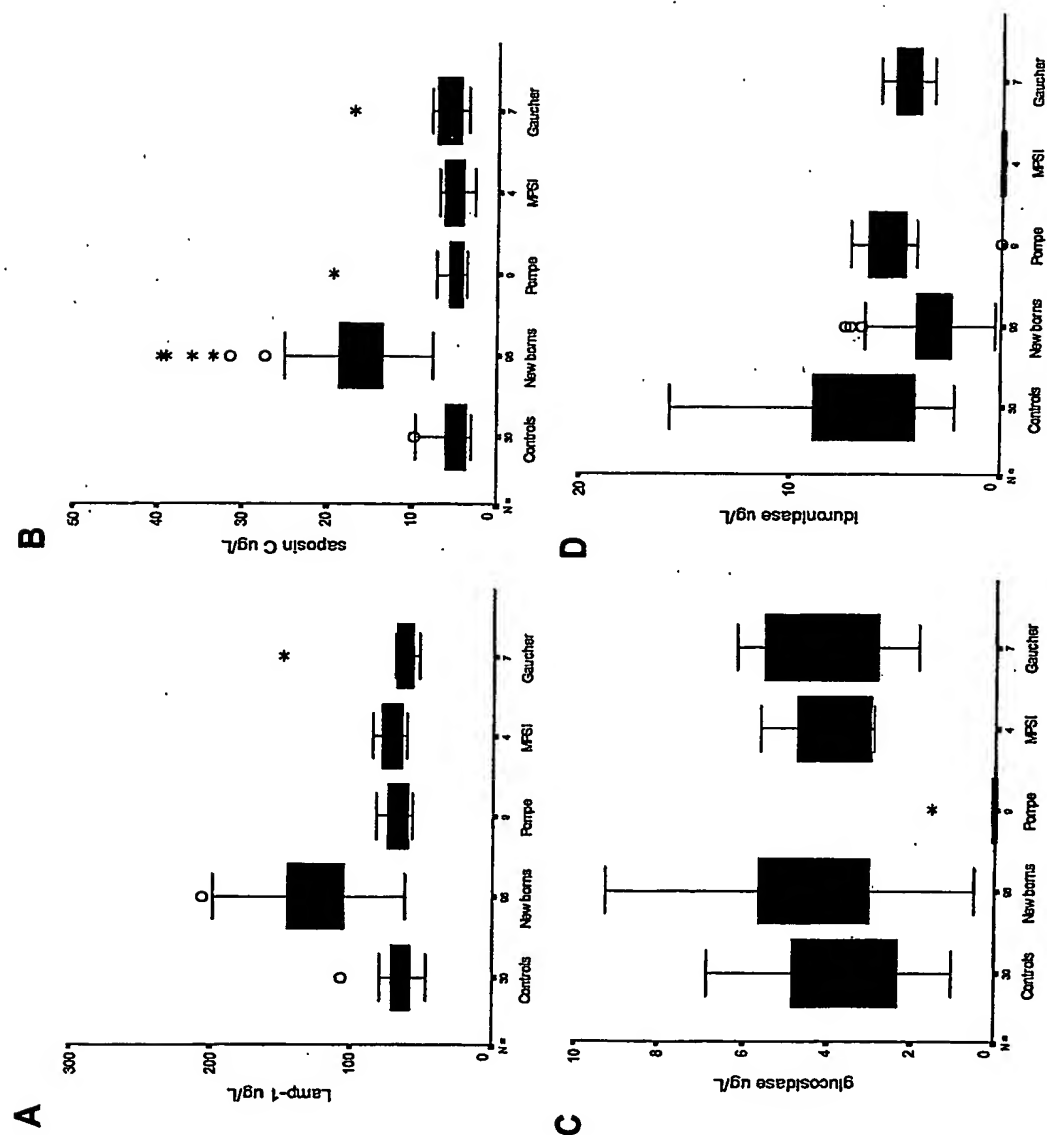


Figure 19

Protein markers for 7-Plex LSD screening

LAMP-1 and saposin C

Disorder	Enzyme Deficiency	Australian Prevalence	Therapy
Gaucher disease	β -glucosidase	1 in 57,000	ERT / BMT
Fabry disease	α -galactosidase A	1 in 117,000	ERT
MPS I	α -L-iduronidase	1 in 88,000	ERT / BMT
Pompe disease	α -glucosidase	1 in 146,000	ERT (trials)
MPS VI	N-acetylgalactosamine 4-sulphatase	1 in 235,000	BMT / ERT (trials)
<div><ul style="list-style-type: none">• Most LSD patients have reduced protein.• Total prevalence detected with 7-plex is 1 in 20,600.</div>			

Figure 20

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Antibody reagents used in 7-plex assays

Assay	Bead region	Capture antibody	$\mu\text{g}/1.25\text{e6}$ beads	Reporter antibody	ng/well
Lamp-1	25	Sheep anti Lamp-1 polyclonal	9	Sheep anti Lamp-1 polyclonal	16
Saposin C	42	Monoclonal 7B2	9	Monoclonal S13C1 G2 G3	8
α -glucosidase	26	Sheep anti α -glucosidase polyclonal	5	Monoclonal 43D1	16
α -Iduronidase	24	Sheep anti α -Iduronidase polyclonal	36	Monoclonal Id1A	16
α -galactosidase	43	Monoclonal AG2.GG9.6.1.6	9	Monoclonal AG2.GF5.1151	32
β -glucosidase	45	Sheep anti β -glucosidase polyclonal	9	Sheep anti β -glucosidase polyclonal	32
N-acetyl-galactosamine-4-sulphatase	46	Sheep anti 4-sulphatase polyclonal	9	Sheep anti 4-sulphatase polyclonal	32

Figure 21

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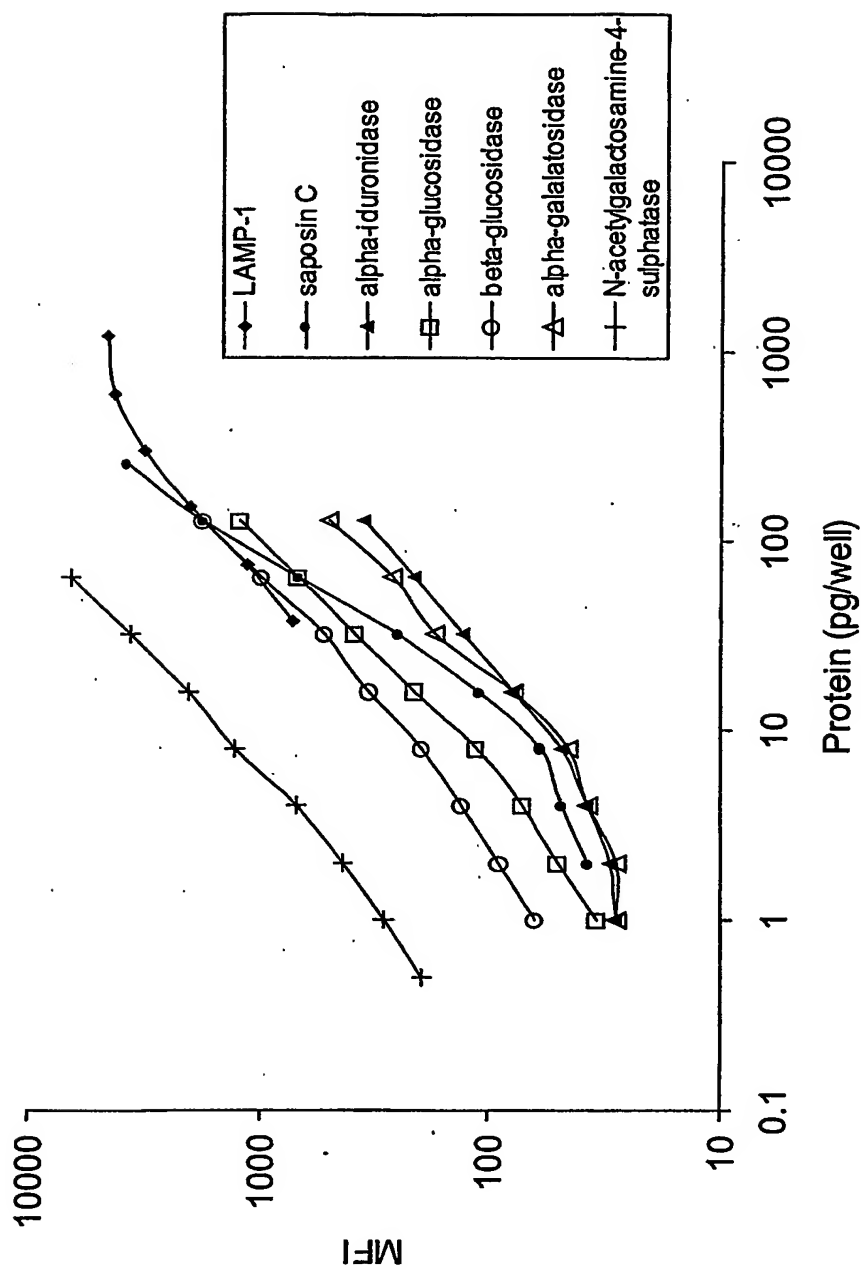


Figure 22

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Adult control protein values in 7-plex assays

Sample ID	Age years	LAMP-1 ng/mL	Saposin C ng/mL	α -iduronidase ng/mL	α -glucosidase ng/mL	β - glucosidase ng/mL	α -galacto sidase ng/mL	N-acetyl galactosamine- 4-sulphatase ng/mL
LDRU C7 EDTA	39.1	32.9	12.7	5.0	7.7	3.3	4.6	0.9
LDRU C9 EDTA	44.1	36.4	10.7	4.8	4.0	3.0	4.2	1.5
LDRU C11 EDTA	43.2	34.5	8.9	7.5	8.2	3.5	4.6	1.2
LDRU C12 EDTA	47.2	28.7	13.2	10.0	8.8	4.1	7.4	1.7
LDRU C13 EDTA	25.2	36.5	14.0	6.3	9.2	3.0	5.9	0.9
LDRU C14 EDTA	22.8	38.5	22.3	7.9	14.0	6.4	8.5	2.3
LDRU C15 EDTA	32.3	38.8	13.5	11.2	10.8	5.3	4.6	1.5
LDRU C16 EDTA	23.9	31.0	12.0	4.9	9.4	3.5	2.7	1.3
LDRU C17 EDTA	24.8	34.7	13.0	8.2	5.6	4.4	4.2	1.1
LDRU C18 EDTA	26.3	29.1	12.1	4.3	5.3	4.0	5.1	1.1
LDRU C19 EDTA	39.8	36.2	13.0	5.9	6.9	3.7	3.6	1.7
LDRU C20 EDTA	31.8	40.9	17.6	8.5	10.1	5.5	7.2	1.8
Average	33.4	34.9	13.6	7.0	8.3	4.1	5.2	1.4
SDev	8.9	3.8	3.4	2.2	2.7	1.1	1.7	0.4
Min	22.8	28.7	8.9	4.3	4.0	3.0	2.7	0.9
Max	47.2	40.9	22.3	11.2	14.0	6.4	8.5	2.3
SDev (MOM)	0.3	0.1	0.3	0.3	0.3	0.3	0.3	0.3
Min (MOM)	0.7	0.8	0.7	0.6	0.5	0.7	0.5	0.6
Max (MOM)	1.4	1.2	1.6	1.6	1.7	1.5	1.6	1.6

Figure 23

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Sample ID	Age years	LAMP-1 ng/mL	Saposin C ng/mL	α -Iduronidase ng/mL	α -glucosidase ng/mL	β -glucosidase ng/mL	α -galactosidase ng/mL	N-acetyl galactosamine-4 sulphatase ng/mL
Newborn 1		23.3	4.1	1.4	1.1	1.5	1.0	0.2
Newborn 2		24.9	3.9	1.8	1.4	1.3	1.3	0.2
Newborn 3		65.1	36.7	4.0	3.7	2.7	6.5	2.2
Newborn 4		72.1	58.2	1.7	12.1	5.2	13.5	2.2
Newborn 5		54.2	56.4	2.1	12.5	4.5	9.6	2.8
Newborn 6		69.0	36.6	0.9	10.2	4.1	17.8	2.0
Newborn 7		44.7	30.3	1.8	6.6	3.7	6.4	3.5
Newborn 8		19.1	3.7	1.4	1.0	0.8	1.0	0.2
Newborn 9		42.5	19.8	3.3	3.8	3.1	5.5	1.6
Newborn 10		52.6	43.9	4.1	9.4	3.9	6.6	2.4
Newborn 11		20.9	3.9	1.8	1.1	1.1	0.9	0.2
Newborn 12		36.8	34.1	1.4	6.6	2.9	5.8	2.7
Newborn 13		56.2	64.4	2.9	13.6	5.6	15.9	3.8
Newborn 14		70.5	55.7	2.8	12.9	5.0	14.4	2.7
Newborn 15		81.1	45.6	1.6	8.3	4.1	18.5	1.9
Newborn 16		63.9	54.8	3.0	12.8	5.7	12.6	2.5
Newborn 17		83.7	72.2	2.6	13.3	6.4	29.0	3.3
Newborn 18		64.8	58.4	3.9	9.4	5.7	14.3	4.8
Newborn 19		82.4	67.8	3.4	9.6	6.6	71.0	3.3
Newborn 20		69.1	61.6	5.7	21.0	6.5	14.6	3.7
Newborn 21		47.2	74.2	3.6	9.1	3.1	8.0	1.5
Newborn 22		63.2	83.9	5.6	16.7	6.4	16.6	4.8
Newborn 23		60.2	43.3	2.9	7.1	4.6	11.1	3.0
Newborn 24		70.7	31.7	2.7	11.9	4.2	22.4	2.1
Newborn 25		62.7	35.8	3.5	10.0	3.6	10.9	2.5
Newborn 26		82.4	64.5	3.4	10.1	5.4	26.9	3.3
Newborn 27		60.7	32.1	3.2	9.6	4.1	12.7	1.9
Newborn 28		86.5	74.4	2.2	18.8	16.4	72.2	4.7
Average		58.2	42.9	2.8	9.4	4.6	16.0	2.5
StDev		19.6	22.8	1.2	5.1	2.9	17.3	1.3
Min		19.1	3.7	0.9	1.0	0.8	0.9	0.2
Max		86.5	83.9	5.7	21.0	16.4	72.2	4.8
StDev(MOM)		0.3	0.5	0.4	0.5	0.6	1.1	0.5
Min (MOM)		0.3	0.1	0.3	0.1	0.2	0.1	0.1
Max (MOM)		1.5	2.0	2.0	2.2	3.6	4.5	1.9

Figure 24

Newborn control protein values

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Pearson correlation coefficients for protein markers in dried blood spots from newborns.

	LAMP-1	Saposin C	α -iduronidase	α -glucosidase	β -glucosidase	α -galactosidase	N-acetylglucosamine-4-sulphatase
LAMP-1	1.00	0.82 ^a	0.31	0.73 ^a	0.70 ^a	0.69 ^a	0.68 ^a
Saposin C	0.82 ^a	1.00	0.47	0.85 ^a	0.75 ^a	0.61 ^a	0.88 ^a
α -iduronidase	0.31	0.47 ^b	1.00	0.48 ^a	0.22	0.09	0.51 ^a
α -glucosidase	0.73 ^a	0.85 ^a	0.48 ^a	1.00	0.77 ^a	0.51 ^a	0.77 ^a
β -glucosidase	0.70 ^a	0.75 ^a	0.22	0.77 ^a	1.00	0.81 ^a	0.74 ^a
α -galactosidase	0.69 ^a	0.61 ^a	0.09	0.51 ^a	0.81 ^a	1.00	0.52 ^a
N-acetylglucosamine-4-sulphatase	0.68 ^a	0.88 ^a	0.51 ^a	0.77 ^a	0.74 ^a	0.52 ^a	1.00

^a p<0.01^b p<0.05

Figure 25

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Adult Control ^a	Age	LAMP-1 ng/mL	Saposin C ng/mL	α -iduronidase ng/mL	α -glucosidase ng/mL	β -glucosidase ng/mL	α -galactosidase ng/mL	N-acetyl galactosamine-4- sulphatase ng/mL
Average	33.4	34.9	13.6	7.0	8.3	4.1	5.2	1.4
StDev	8.9	3.8	3.4	2.2	2.7	1.1	1.7	0.4
Min	22.8	28.7	8.9	4.3	4.0	3.0	2.7	0.9
Max	47.2	40.9	22.3	11.2	14.0	6.4	8.5	2.3
Patient ^b								
Fabry	38.15	35.55	29.24 ^c	2.85 ^d	6.32	4.19	0.00 ^d	1.95
Fabry	34.86	37.48	27.34 ^c	7.76	9.94	4.30	0.00 ^d	1.32
Fabry	26.95	29.56	8.60 ^d	4.03 ^d	5.92	2.76 ^d	0.00 ^d	0.57 ^d
MPS I	NA	30.01	7.71 ^d	0.27 ^d	4.96	1.21 ^d	1.87 ^d	0.53 ^d
MPS I	0.77	35.74	11.11	0.00 ^d	6.51	2.36 ^d	5.97	1.14
MPS II	3.89	52.15 ^c	37.13 ^c	8.90	8.21	4.04	6.99	2.28 ^c
MPS VI	4.84	40.74	11.76	6.41	7.24	3.45	3.98	0.00 ^d
MPS VI	NA	29.03	11.66	5.35	4.25	1.58 ^d	3.43	0.00 ^d
ML II/II	0.94	44.07 ^c	31.60 ^c	59.02 ^c	27.76 ^c	4.99	3.48	5.10 ^c
ML II/II	1.92	44.69 ^c	74.10 ^c	33.66 ^c	36.38 ^c	8.71 ^c	6.74	9.91 ^c
Pompe		44.83 ^c	16.56	8.49	0.13 ^d	5.55	8.48	1.98
Pompe	39.21	35.89	12.73	6.54	0.19 ^d	2.80 ^d	3.68	2.10
Pompe	24.40	36.61	16.99	3.77 ^d	0.15 ^d	2.43 ^d	3.78	1.47
Pompe	57.80	35.30	20.62	5.42	0.00 ^d	3.89	4.03	1.41
Pompe	10.65	34.75	10.91	2.44 ^d	0.00 ^d	2.65 ^d	12.51 ^c	1.28
Pompe	8.35	34.51	15.93	4.43	0.07 ^d	3.95	2.12 ^d	1.06
Pompe	10.56	44.09 ^c	27.31 ^c	7.21	0.09 ^d	4.46	8.74 ^c	1.67

^a Adult controls (n=12); ^b MPS = mucopolysaccharidosis; ML = mucopolipidosis.^c indicates above control range; ^d indicates below control range

Figure 26

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	Age	LAMP-1	Saposin C	α -iduronidase ng/mL	α -glucosidase ng/mL	β -glucosidase ng/mL	α -galactosidase ng/mL	N-acetyl galactosamine-4- sulphatase ng/mL
Newborn Controls ^a		ng/mL	ng/mL	ng/mL	ng/mL	ng/mL	ng/mL	ng/mL
Average		58.2	42.9	2.8	9.4	4.6	16.0	2.5
StDev		19.6	22.8	1.2	5.1	2.9	17.3	1.3
Min		19.1	3.7	0.9	1.0	0.8	0.9	0.2
Max		86.5	83.9	5.7	21.0	16.4	72.2	4.8
Patient ^b								
Fabry	38.15	35.55	29.24	2.85	6.32	4.19	0.00 ^d	1.95
Fabry	34.86	37.48	27.34	7.76 ^c	9.94	4.30	0.00 ^d	1.32
Fabry	26.95	29.56	8.60	4.03	5.92	2.76	0.00 ^d	0.57
MPS I	NA	30.01	7.71	0.27 ^d	4.96	1.21	1.87	0.53
MPS I	0.77	35.74	11.11	0.00 ^d	6.51	2.36	5.97	1.14
MPS VI	4.84	40.74	11.76	6.41 ^c	7.24	3.45	3.98	0.00 ^d
MPS VI	NA	29.03	11.66	5.35	4.25	1.58	3.43	0.00 ^d
ML II/III	0.94	44.07	31.60	59.02 ^c	27.76 ^c	4.99	3.48	5.10 ^e
ML II/III	1.92	44.69	74.10	33.66 ^c	36.38 ^c	8.71	6.74	9.91 ^c
Pompe		44.83	16.56	8.49 ^c	0.13 ^d	5.55	8.48	1.98
Pompe	39.21	35.89	12.73	6.54 ^c	0.19 ^d	2.80	3.68	2.10
Pompe	24.40	36.61	16.99	3.77	0.15 ^d	2.43	3.78	1.47
Pompe	57.80	35.30	20.62	5.42	0.00 ^d	3.89	4.03	1.41
Pompe	10.65	34.75	10.91	2.44	0.00 ^d	2.65	12.51	1.28
Pompe	8.35	34.51	15.93	4.43	0.07 ^d	3.95	2.12	1.06
Pompe	10.56	44.09	27.31	7.21 ^c	0.09 ^d	4.46	8.74	1.67

^a Newborn controls (n=28); ^b MPS = mucopolysaccharidosis; ML = mucopolipidosis.^c indicates above control range; ^d indicates below control range

Figure 27

Multiplex neonatal screening strategy for LSD

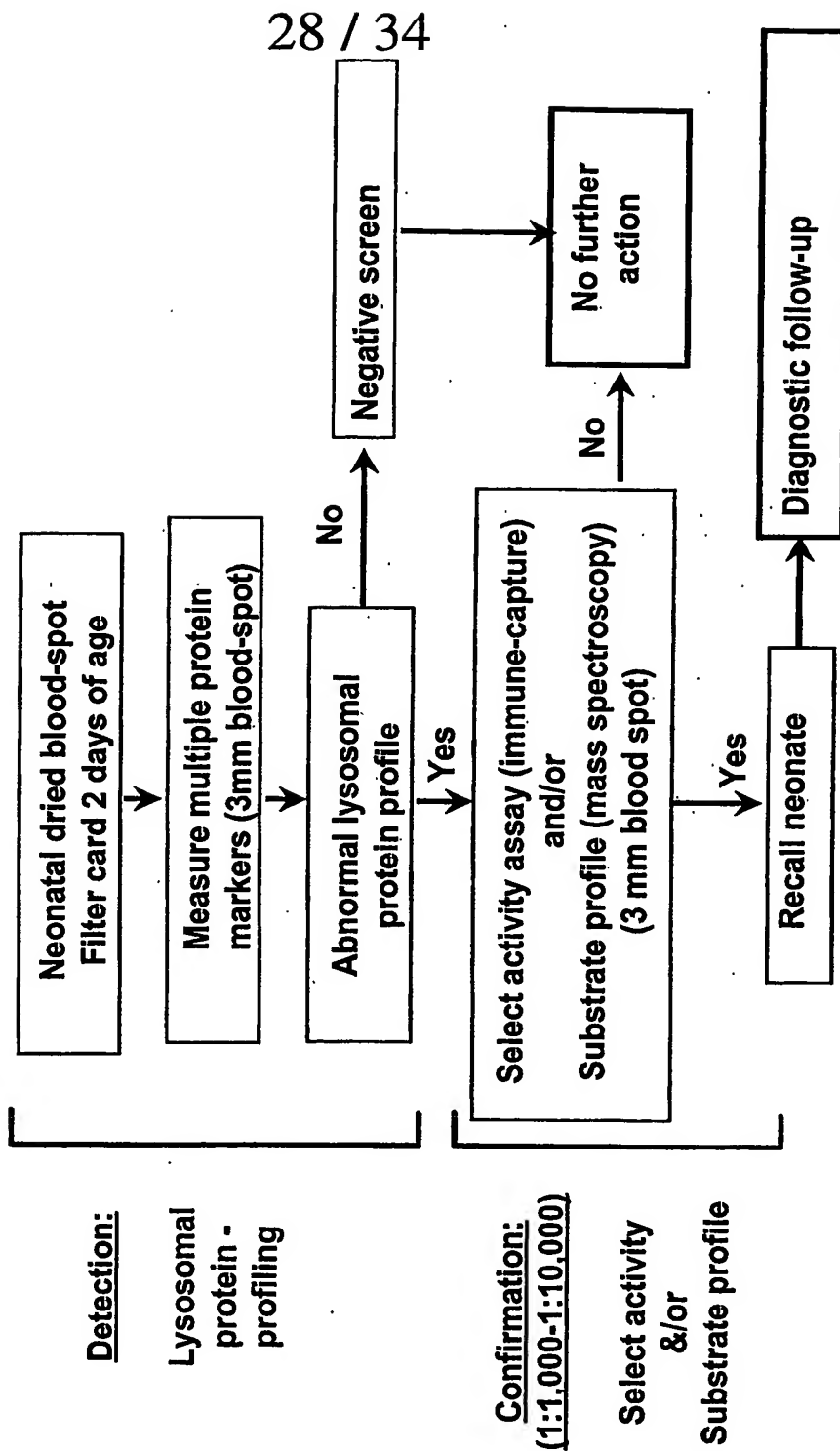


Figure 28

Derivatisation of oligosaccharides for MS/MS

1-phenyl-3-methyl-5-pyrazolone (PMP)

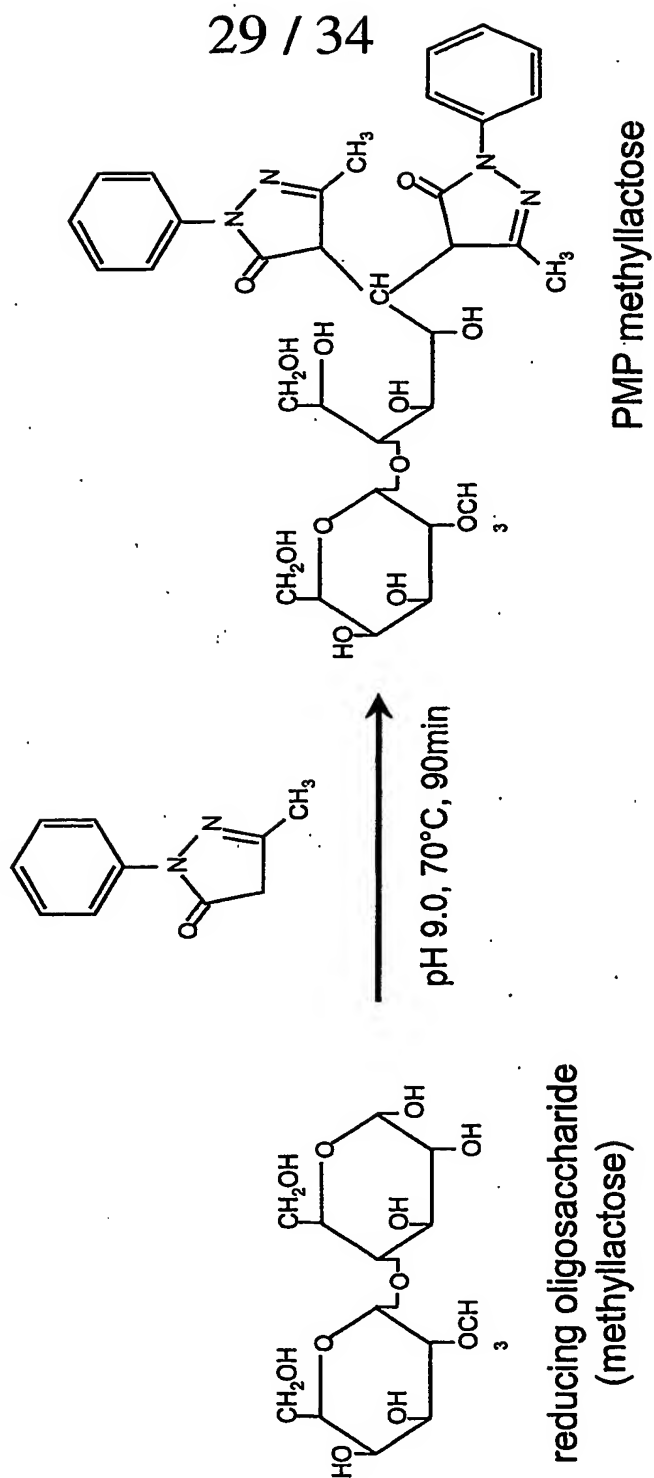


Figure 29

MSMS analysis of α -mannosidosis urine
(Precursor ion scan of m/z 175)

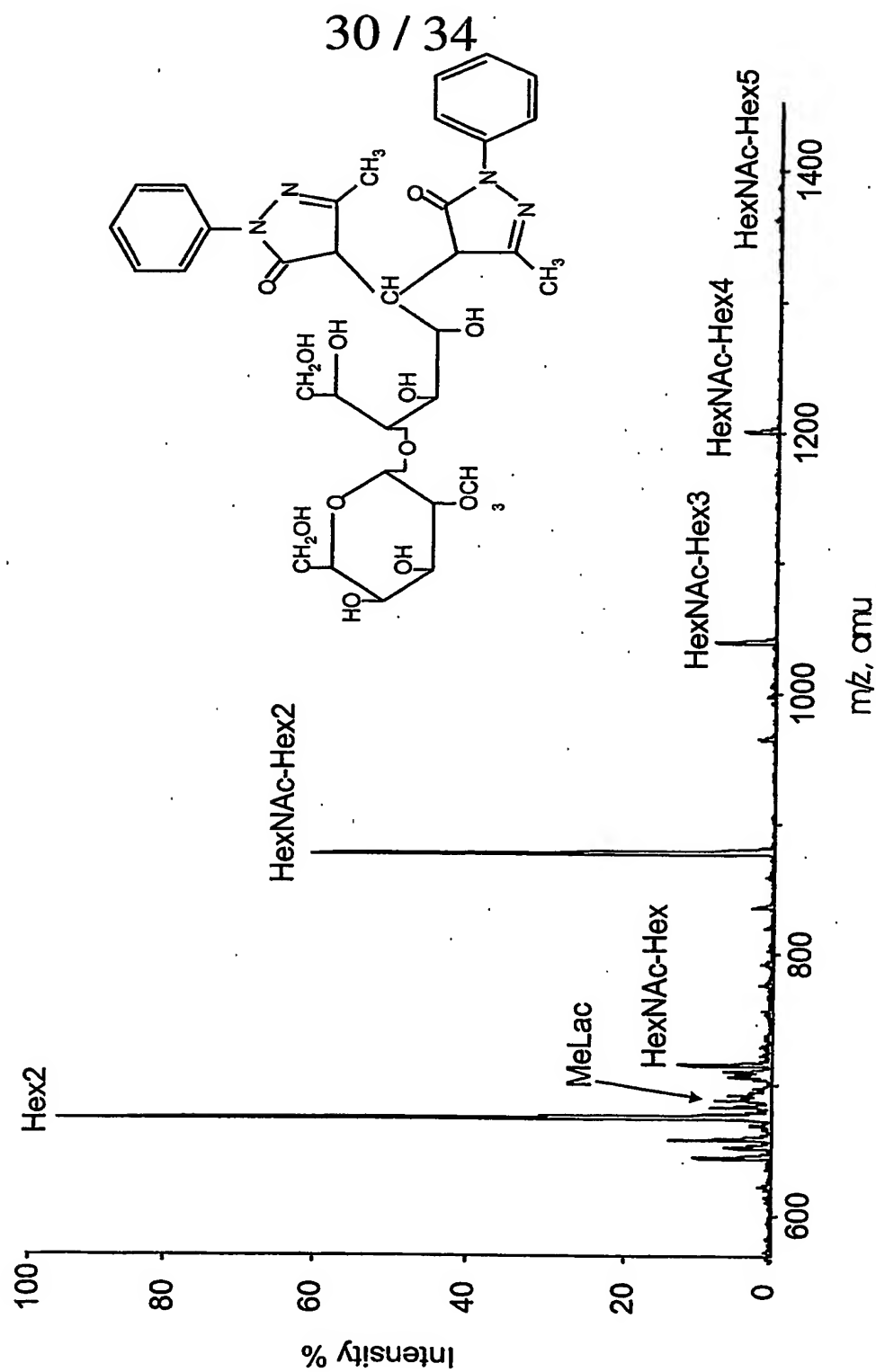
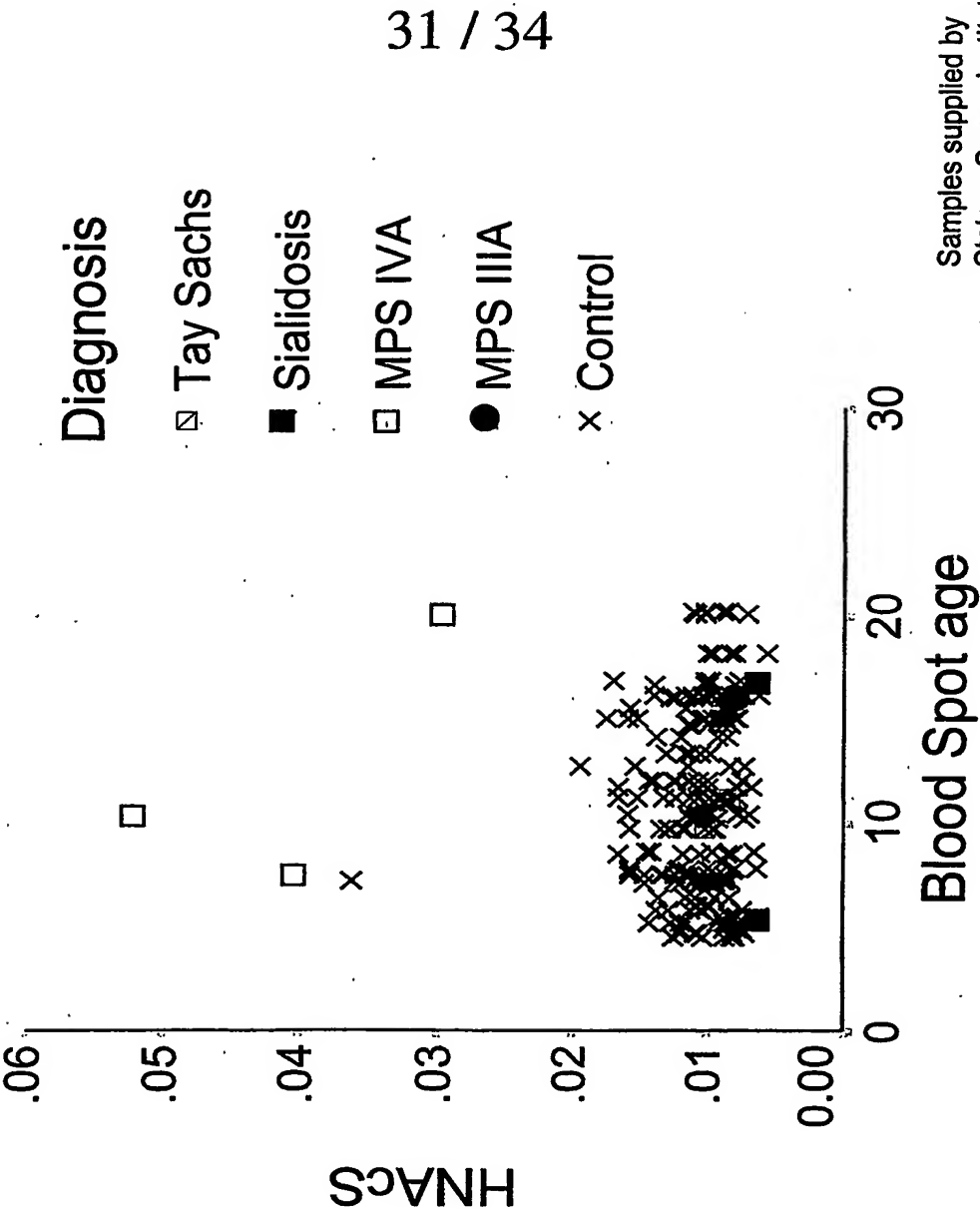


Figure 30

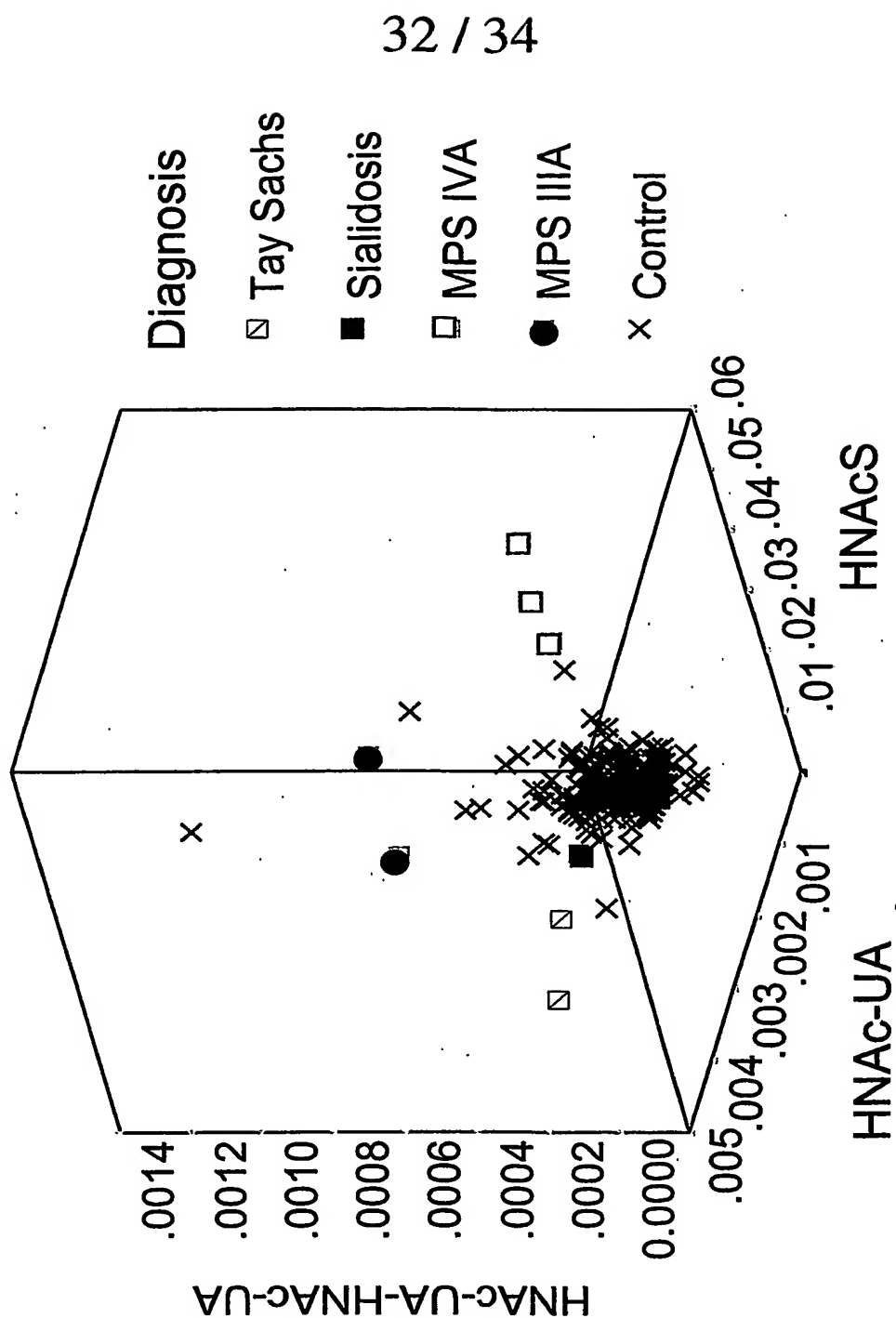
Retrospective analysis of newborn blood spots



Samples supplied by
Statens Serum Institut,
Copenhagen, Denmark

Figure 31

Retrospective analysis of newborn blood spots



Samples supplied by
Statens Serum Institut,
Copenhagen, Denmark

Figure 32

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Summary of retrospective analysis of newborn blood spots

Disorder	n	Markers	Sensitivity/ Specificity
α -Mannosidosis	1	H2/HNAc	100 / 99.6
MPS II	4	-	-
MPS IIIA	2	HNAc-UA-HNAc-UA	100 / 100
MPS IVA	3	HNAcS	100 / 100
I-cell disease	2	GC/LC	100 / 100
Sialidosis	3	HNS-UA	67 / 100
Pompe disease	3	-	-
Sandhoff disease	6	-	-
Tay-Sachs disease	2	HNAc-UA	100 / 99.6

Figure 33

Protein markers for LSD screening

Disorder	Enzyme Deficiency	Australian Prevalence	Therapy
Gaucher disease	β -glucosidase	1 in 57,000	ERT
Fabry disease	α -galactosidase A	1 in 117,000	ERT
MPS I	α -L-iduronidase	1 in 88,000	ERT
Pompe disease	α -glucosidase	1 in 146,000	ERT (trials)
MPS VI	N-acetylglucosamine 4-sulphatase	1 in 235,000	ERT (trials)
MPS II	iduronate-2-sulphatase	1 in 136,000	ERT (trials)
Krabbe disease	galactocerebrosidase	1 in 201,000	BMT
MLD	arylsulphatase A	1 in 92,000	BMT
MPS IVA	galactose 6-sulphatase	1 in 169,000	ERT (proposed)
Niemann-Pick type A/B	acid sphingomyelinase	1 in 248,000	ERT (proposed)
MPS IIIA	heparan-N-sulphatase	1 in 114,000	Research
MPS IIIB	α -N-acetylglucosaminidase	1 in 211,000	Research
TOTAL (n = 12)		1 in 10,500	

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Figure 34